


Male meiotic recombination rate varies with seasonal temperature fluctuations in wild populations of autotetraploid *Arabidopsis arenosa*

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Abstract

Meiosis, the cell division by which eukaryotes produce haploid gametes, is essential for fertility in sexually reproducing species. This process is sensitive to temperature, and can fail outright at temperature extremes. At less extreme values, temperature affects the genome-wide rate of homologous recombination, which has important implications for evolution and population genetics. Numerous studies in laboratory conditions have shown that recombination rate plasticity is common, perhaps nearly universal, among eukaryotes. These studies have also shown that variation in the length or timing of stresses can strongly affect results, raising the important question whether these findings translate to more variable field conditions. Moreover, lower or higher recombination rate could cause certain kinds of meiotic aberrations, especially in polyploid species—raising the additional question whether temperature fluctuations in field conditions cause problems. Here, we tested whether (1) recombination rate varies across a season in the wild in two natural populations of autotetraploid *Arabidopsis arenosa*, (2) whether recombination rate correlates with temperature fluctuations in nature, and (3) whether natural temperature fluctuations might cause meiotic aberrations. We found that plants in two genetically distinct populations showed a similar plastic response with recombination rate increases correlated with both high and low temperatures. In addition, increased recombination rate correlated with increased multivalent formation, especially at lower temperature, hinting that polyploids in particular may suffer meiotic problems in conditions they encounter in nature. Our results show that studies of recombination rate plasticity done in laboratory settings inform our understanding of what happens in nature.

KEYWORDS

evolution, meiosis, plasticity, polyploid, recombination

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1 | INTRODUCTION

Among sexual eukaryotes, meiosis has both functional and evolutionary importance. Meiosis is the specialized cell division by which eukaryotes create haploid gametes. It is essential for fertility in almost all eukaryotic species. An important aspect of meiosis is homologous recombination, the exchange of genetic material among the two parental chromosome copies (Hunter, 2015; Zickler & Kleckner, 1999, 2015). Homologous recombination events are important for two major reasons: First, they are essential (in most species) for the physical process of chromosome segregation (Hunter, 2015; Jones & Franklin, 2006; Zickler & Kleckner, 1999, 2015). This is because recombination events, which develop into cytologically visible chiasmata by metaphase I, establish connections among chromosomes that generate tension on the spindle when homologous centromeres are oriented towards opposing poles. This tension is important for the progression of meiosis and to allow the reliable segregation of homologues to opposing poles in meiosis I (Jones & Franklin, 2006). Second, recombination events also shuffle DNA among homologous chromosome copies, generating novel allele combinations that selection can act upon, with important implications for inheritance, population genetics, and adaptive evolution, as well as genome-wide patterns of for example, genetic load and diversity (Barton, 1995, 2009; Barton & Charlesworth, 1998; Dapper & Payseur, 2017; Feldman et al., 1996; Felsenstein, 1974; Otto & Lenormand, 2002). Recombination rate is thus an important parameter in evolutionary modeling and population genetic analyses. More recombination means more genetic shuffling, and this can result in faster adaptation due to more efficient breakdown of deleterious linkages, effectively “freeing” alleles from linked deleterious variants (Feldman et al., 1996; Felsenstein, 1974; Otto & Lenormand, 2002). On the other hand, recombination is mutagenic and/or can break down positive associations of genes (Arbeithuber et al., 2015; Barton & Charlesworth, 1998; Guirouilh-Barbat et al., 2014; Halldorsson et al., 2019; Reeve et al., 2016). Thus, recombination has both costs and benefits.

Recombination rates are known to vary widely among species, populations, even individuals throughout eukaryotes (Johnston et al., 2016, 2018; Kong et al., 2014; Stapley et al., 2017). However, recombination rate is also not a static parameter; genome-wide recombination rates are known from laboratory studies in a wide range of organisms to be plastic to environmental conditions (e.g., Aggarwal et al., 2019; Bomblies et al., 2015; Kohl & Singh, 2018; Lloyd et al., 2018; Modliszewski et al., 2018; Morgan et al., 2017; Plough, 1917; Rybnikov et al., 2017, 2020). Although multiple environmental factors can affect recombination rate, temperature has a particularly strong effect. Over the reproductive lifespan of individuals, recombination rate is generally a reversible trait, though at the individual cell level it almost certainly is not. Plasticity of recombination to temperature has been reported almost everywhere it has been looked for, and in a wide range of eukaryotes there is a U-shaped relationship, meaning that both high and low temperatures

relative to some species-specific mid-point can cause increased recombination rate (reviewed in Bomblies et al., 2015).

Theoretical studies have made the case that recombination rate plasticity can, in at least some cases, be adaptive (Agrawal et al., 2005; Hadany & Beker, 2003), though the conditions where it is so, are more restrictive in diploids than in haploids (Agrawal et al., 2005). While recombination rate plasticity seems to vary quantitatively, the observation of plasticity in almost all systems suggests there is something truly fundamental about it that may link to the core mechanics of meiosis, which would suggest that rather than “adaptive plasticity”, recombination rate increases may be early symptoms of meiosis beginning to go awry (Morgan et al., 2017). One key link may be to cellular stress. Recombination rates are affected by proteins, such as the cohesins, that in *Drosophila* have been shown to be sensitive to the oxidative state of a cell (Perkins et al., 2016), which is correlated with organismal stress. Indeed, several studies have shown that nonadapted (thus more stressed) individuals have a stronger plastic recombination response to a particular condition than unstressed or adapted individuals; this has been most extensively explored in insects, but is also reported in plants (Aggarwal et al., 2019; Bomblies et al., 2015; Buss & Henderson, 1988; Rybnikov et al., 2017, 2020; Shaw, 1972). Temperature affects meiosis-specific structures called the chromosome axis and synaptonemal complex in a wide range of eukaryotes (Bomblies et al., 2015; Lloyd et al., 2018), and the length of these structures correlates positively with recombination rate (Kleckner et al., 2003). Temperature also affects chromatin structure, and mutation in chromatin remodelling genes can alter the recombinational response to temperature in *Arabidopsis thaliana* (Choi et al., 2013). Accordingly, early stages in meiotic prophase I where recombination events are initiated and develop (Zickler & Kleckner, 1999, 2015), seem to be particularly sensitive to temperature perturbation (De Storme & Geelen, 2020; Draeger & Moore, 2017).

Though the phenomenon of recombination rate plasticity has been known about for over a century (Plough, 1917), our understanding of its role in evolution remains in its early stages. For good reasons, recombination rate plasticity has been studied almost exclusively in a laboratory context, where temperature and other conditions can be tightly controlled (see for review: Bomblies et al., 2015; Wilson, 1959). Although plasticity is commonly reported in laboratory studies, it has also become clear from these studies that experimental design, such as timing of the heat treatment relative to recombination assay, nature of the heat treatment (e.g., short vs. long exposures, slightly elevated or extreme temperatures), and suddenness of the change (e.g., gradual vs. sudden rise in temperature; Bomblies et al., 2015; De Storme & Geelen, 2020; Wilson, 1959), has a large influence over whether plasticity is observed, which direction the effects go in, and their strength. It thus remains unclear how recombination rate might respond in the more variable and unpredictable conditions organisms experience in nature during a growing season, or to what extent laboratory results connecting temperature

to recombination rate variation translate to natural conditions in the field. To address this, we studied recombination rate variation cytologically in two wild autotetraploid populations of *Arabidopsis arenosa* across a growing season in Switzerland.

In addition to the effects noted above, low or high recombination rates can, in some cases, be associated with aberrations in chromosome segregation. Excessively low genome-wide recombination rates can cause some chromosomes to have no recombination, leading to univalents and thus aneuploidy (Jones & Franklin, 2006). In some systems, elevated recombination may also be immediately costly. While in *A. thaliana* higher recombination rates due to overexpression of a crossover-promoting factor (HEI10) and deletion of crossover suppressors (RECQ4A and RECQ4B), are generally not linked to obvious problems in the short term (Serra et al., 2018), yeast mutants for SGS1 (another RecQ-related gene) have a hyper-recombination phenotype that correlates with frequent chromosome mis-segregation (Watt et al., 1995). In Autopolyploids, which arise from within-species whole genome duplication and thus have multiple equally homologous chromosomes that all have the potential to recombine (Bomblies & Madlung, 2014; Otto & Whitton, 2000; Ramsey & Schemske, 1998), there is an additional danger. We previously hypothesized based on theoretical arguments that autopolyploids might be particularly sensitive to temperature effects (as discussed in Bomblies et al., 2015). This is because, in these species, lower recombination rates may help prevent the formation of multivalents, which are deleterious associations of more than two homologues that can lead to chromosome mis-segregation (Bomblies et al., 2016; Grandont et al., 2013). If autopolyploid species experience increased recombination due to changes in temperature, it may be that they will start forming multivalent associations at higher rates. Thus, plastic increases of recombination rate in response to high or low temperature might pose unique challenges for autopolyploids.

Here, we undertook a cytological study of male meiosis in two genetically distinct natural autotetraploid populations of *A. arenosa* across a growing season in Switzerland. We tracked fine-scale changes in temperature with multiple temperature sensors in each site, and sampled flower buds at three timepoints that spanned the duration of the flowering season. We used metaphase spreads to cytologically estimate recombination rates from wild plants, and detect any abnormalities that might contribute to chromosome mis-segregation. We had four main aims: (1) to ask whether recombination rates vary across a growing season in nature, thus causing individuals to produce gametes with a range of genetic shuffling; (2) to test whether this correlates with temperature as is observed in laboratory conditions; (3) to ask whether the temperature ranges experienced in natural conditions are sufficient to cause aberrations such as unpaired univalents, or a higher rate of multivalent formation, and (4) to ask whether results from laboratory studies of related species, *A. thaliana* in this case (Lloyd et al., 2018; Modliszewski et al., 2018), are informative for the much more variable conditions plants experience in nature. We found that the answer to all four questions is yes, although the effects vary quantitatively.

2 | MATERIALS AND METHODS

2.1 | Sites

Site locations were two roadside alpine meadow sites in the Alps, in Göschenertal in Kanton Uri, Switzerland (N46.66056, E8.53859 and N46.660335, E8.535261), and three limestone rock outcrop sites in the Jura mountains, from the Gorges de Court at Moutier (Kanton Bern), Switzerland (N47.262056, E7.350866; N47.2585425, E7.3460552; and N47.246476 E7.346452). Here, we abbreviate Göschenen as “GOS” and Moutier as “MOU”. Sites were initially identified from searches of *Arabidopsis arenosa* samples in the online collection of the University of Zürich/ETH herbaria (<https://www.herbarien.uzh.ch/en.html>).

2.2 | Temperature sensors, rainfall data, developmental age, and sampling regime

To measure changes in environmental temperature throughout the flowering season, we deployed iButton Thermochron sensors (model DS1921G, maxim integrated) among groups of *A. arenosa* plants at the onset of budding in each site. These sensors measured temperature hourly through the study period at an accuracy of $\pm 1^\circ\text{C}$. All iButtons were housed inside PVC radiation shields following guidelines given in Terando et al. (2017) to minimize biases in temperature measurements. Rainfall data were gathered from stations of the Federal Office of Meteorology and Climatology MeteoSwiss (GOS station location: $8^\circ 35' 43''\text{E}$, $46^\circ 41' 43''\text{N}$; MOU station location: $7^\circ 22' 18''\text{E}$, $47^\circ 16' 20''\text{N}$). Developmental age was estimated by proxy by counting the number of flowers and fruits already developed per sampled inflorescence. Within each of the three sites at MOU, five individual flowering plants of approximately the same size and age were labelled for repeated collection throughout the study. Within each of the two sites at GOS, six individuals were likewise labelled for repeated sampling using these same criteria. We sampled buds at three timepoints per site that spanned the duration of the flowering season, each separated by 3 weeks. Bud samples from each labelled individual in both sites were collected around midday in each of these three sampling timepoints using forceps, and immediately stored in a 3:1 fixative solution of ethanol to acetic acid for cytological processing in the laboratory. All data are summarized in Table S1, and are provided in detail via the ETH Research Collection under <https://doi.org/10.3929/ethz-b-000471742>

2.3 | Cytology and microscopy

Buds collected from labelled individuals were used to create a series of metaphase I spreads from male meiocytes via enzyme digestion and DAPI staining following Morgan et al. (2020). Briefly, buds were removed from their fixative solution, washed in a 0.01 M citrate buffer solution, and digested in a pectolyase-cellulase medium.

Digested buds were then macerated on microscope slides and mounted with cover slips using a DAPI and VECTASHIELD solution. Slides with meiocytes in metaphase I were imaged at 100 \times using a Zeiss Axio Imager 2 microscope interfaced with a Zeiss AxioCam MRm monochrome camera. We examined all images of these metaphase spreads and filtered by quality by using only those cells for crossover counts where we could confidently assign a shape to 10 or more bivalents. We quantified crossover numbers for each cell from the shape of bivalents ("ring" bivalents have 2COs, "rod or cross-shaped bivalents" have 1CO, chain quadrivalents have three COs, and ring quadrivalents have 4) as previously defined for *A. thaliana* (Sanchez Moran et al., 2001) and *A. arenosa* (Morgan et al., 2020). Crossover rates per chromosome were then calculated by dividing the total crossovers counted per cell by the total "scorable" chromosomes (calculated as $2 \times$ number of bivalents + $1 \times$ number of univalents + $4 \times$ number of multivalents) from which crossovers could be counted. Likewise, univalent rates and multivalent rates were calculated by dividing their total counts per cell by the total "scorable" chromosomes from the same cell. All images are freely available under <https://doi.org/10.3929/ethz-b-000471742>

2.4 | Statistical analyses

All statistical analyses and figures were produced using the R statistical platform (version 4.0.3) via R STUDIO software (version 1.3.1093). All plots were generated using the GGLOT2 package (Wickham, 2009) with data manipulated via the DPLYR package (Wickham et al., 2017). The RASTER package (Hijmans, 2016) was used to generate a map of Switzerland shaded by a 90 m resolution elevation layer, labelled with our two study sites.

To test for relationships between crossover, univalent, and multivalent rates in each labelled plant with seasonal changes in ambient temperature, we isolated a 30-h window of temperatures prior to the time buds were collected from each plant. Temperatures within this window were isolated using the hourly temperature measurements that were recorded among labelled plants in each site. Since this window more than fully encompasses premeiosis to prophase I to metaphase I in *A. thaliana* (Armstrong et al., 2003), we chose five temperature periods within it for our statistical comparisons: 30 h prior to bud collection, 20 h prior to bud collection, 10 h prior to bud collection, the time of bud collection, and the average temperature 10–30 h prior to bud collection. We then built a series of polynomial regression models using the lm() function in R, where the response variables to each temperature period were averaged "per chromosome" crossover rate, univalent rate, and multivalent rate values. These averaged values were calculated across all cells scored within each labelled plant for each of the three bud-collection timepoints by dividing their total counts by the total number of scorable chromosomes. Each polynomial regression model was structured to test the relationship between crossover rates, univalent rates, and multivalent rates as independent response variables to each temperature period as separate explanatory variables, with sites as the

main interacting coefficient with temperature in each model. With this model structure, we are able to test for significant site-specific differences in the slope of each explanatory to response variable relationship. The summary() function in R was used to generate significance summaries for each model. Significance tests for the main site interaction in each model were performed using type III ANOVA via the Anova() function in the CAR package (Fox & Weisberg, 2011), as type III ANOVA is required to properly account for interactions among model coefficients.

To test for overall relationships between crossover, univalent, and multivalent rates in each labelled plant, we built two linear regression models using the lm() function in R. These models were structured with both response variables as per cell crossover rates, and each independent explanatory variable as per cell univalent and multivalent rates. As with our polynomial regression models, site was included as the main interacting coefficient in both of these models to test for significant site-specific differences in the slope of each explanatory to response variable relationship. Likewise, the summary() function in R was used to generate significance summaries for each model, and type III ANOVA was applied for significance tests of the main site interaction in each model.

Lastly, to test for site-specific differences in average crossover, univalent, and multivalent rates through each sampling timepoint, we fit a series of analysis of variance models using the aov() function in R. These models were structured to test the relationship between averaged crossover, univalent, and multivalent rates as independent response variables to sampling time and sites as two independent explanatory variables. Interactions between sampling time and sites were also included in each model to test for significant site- and timepoint-specific differences in the values of each explanatory to response variable relationship. Again, the summary() function was used to generate significance summaries for each model, and significance tests for the main effects of site and timepoint as well as the site:timepoint interaction were performed using type III ANOVA via the Anova() function.

2.5 | Population genetic analyses

Because there could be differences arising from genotype and population history, and because some *A. arenosa* lineages are especially stress resilient (Baduel et al., 2016), we needed to know which lineages the sampled plants belong to in order to contextualize our results. To quantify genetic relatedness, we used 182 published genome sequences (Monnahan et al., 2019) sampled across Europe, and 10 new individuals sampled in GOS and MOU. For whole genome sequencing of the five MOU and five GOS plants, young leaves were dried, DNA was extracted using the NucleoSpin Plant II kit (Macherey-Nagel), libraries were prepared (Illumina TruSeq DNANano) and sequenced (Illumina Novaseq, paired-end, 40.48 \times mean coverage). We aligned raw data (available at NCBI SRA accession SRP268902) to the *Arabidopsis lyrata* reference genome (Hu et al., 2011) using bwa mem (Li & Durbin, 2009). We then processed

the data as previously described (Monnahan et al., 2019) using samtools (Li et al., 2009) and called SNPs using GATK 3.7 (Van der Auwera et al., 2013) using the MOU and GOS samples with a callset of 129 diploid *A. arenosa* individuals. The final tetraploid data set was constructed by combining the VCF file from previously called tetraploid samples (Monnahan et al., 2019) and the 10 additional MOU and GOS individuals using bcftools (Li et al., 2009), GATK CombineVariants and filtered according to GATK 3.7 best practices and filters described in (Monnahan et al., 2019). This data set contained 2092672 variant sites. Finally, we pruned this data set based on estimated linkage disequilibrium (LD) using a custom R script (see <https://github.com/LZeitler/tetraploids> window size 1,000, r cutoff 0.1) to 214580 polymorphic sites of tetraploid populations. We calculated principal components as previously described (Monnahan et al., 2019), using a modified version of adegenet::glPca (Jombart, 2008).

3 | RESULTS

3.1 | Göschenen and Moutier represent distinct genetic lineages in *Arabidopsis arenosa*

We sampled two natural autotetraploid *A. arenosa* populations within Switzerland (Figure 1a; see Methods). The *A. arenosa* autotetraploids, while all monophyletic with respect to diploids, have separated since their origin about 30,000 generations ago into at least five genetically distinct lineages (Arnold et al., 2015; Monnahan et al., 2019). Importantly, plants within one of these lineages respond differently to temperature stress (Badael et al., 2016, 2018). Since stress can play a role in the observation or extent of recombination rate plasticity (Aggarwal et al., 2019; Bombliet et al., 2015; Buss & Henderson, 1988; Rybníkov et al., 2017, 2020; Shaw, 1972), we wished to first ascertain which genetic lineages the included sites originate from in order to contextualize our results. We thus generated whole-genome short read sequencing of five individuals per population and incorporated these into a principal component analysis (PCA) with 182 previously published tetraploid genomes from all known tetraploid *A. arenosa* lineages (Monnahan et al., 2019). We found that the two sites we sampled in Switzerland, from Gorges de Court, in the Bernese Jura, near Moutier, Kanton Bern (MOU), and the Göschenental, in the Alps, near Göschenen, Kanton Uri (GOS), are members of genetically and phenotypically distinct lineages within *A. arenosa* (Figure 1b). The plants from MOU are part of what has previously been named the Swabian or Hercynian lineage (Arnold et al., 2015; Monnahan et al., 2019; Figure 1b), examples of which are also found on geologically contiguous limestone outcrops in forested areas in Southern Germany, and in a band of hill regions north of the Alps from the Czech Republic to Belgium. Plants from Göschenen, on the other hand, are part of a broadly distributed “ruderal” lineage (“ruderal” is defined as plants growing on human-disturbed land) found especially in railways, but also roadsides, throughout much of northern and central Europe (Arnold

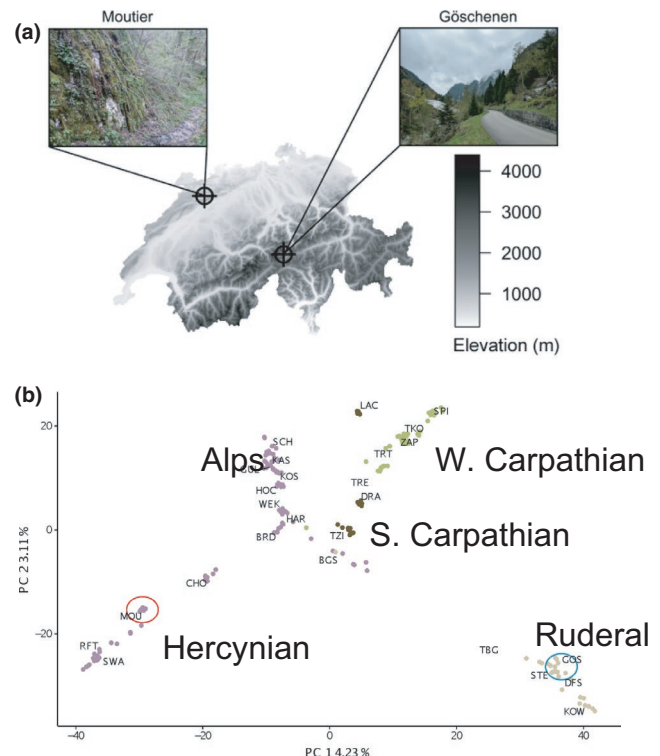


FIGURE 1 Sampling sites and genetic relatedness of populations. (a) Sampling site locations in Switzerland, with site photos. See methods for GPS coordinates. (b) Principal component analysis (PCA) of Moutier (MOU) and Göschenen (GOS) in a broader sample of *Arabidopsis arenosa* tetraploids from (Monnahan et al., 2019). GOS is part of a ruderal lineage of *A. arenosa*, which grows primarily on railways and roadsides, clustering with populations from as far away as Sweden (DFS) and Poland (KOW), while MOU clusters with the Hercynian *A. arenosa* from similar limestone forested rock outcrop habitats in southern Germany and the Czech Republic

et al., 2015; Monnahan et al., 2019; Figure 1b). The ruderal lineage has been previously shown to be strongly tolerant of both cold and heat stress, probably due to overexpression of heat shock and cold response genes (Badael et al., 2016), raising the possibility that GOS plants might be less sensitive to climate than MOU plants.

3.2 | Crossover rates in response to temperature

We tracked temperature surrounding sampled plants in both populations (see Methods; Figure 2a), obtained rainfall data, and estimated developmental age of each inflorescence by counting flower and fruit numbers below the sampled buds. We cytologically assessed the male recombination rate for each plant from multiple metaphase I spreads at three timepoints in the season (Table 1; Figure 2a). Since there is substantial variation among cells, we used averaged “per chromosome” recombination rate values calculated from multiple cells for each plant for analysis of temperature effects on recombination rate. We calculated this by dividing the total number of chiasmata we counted per cell by the number of scorable chromosomes

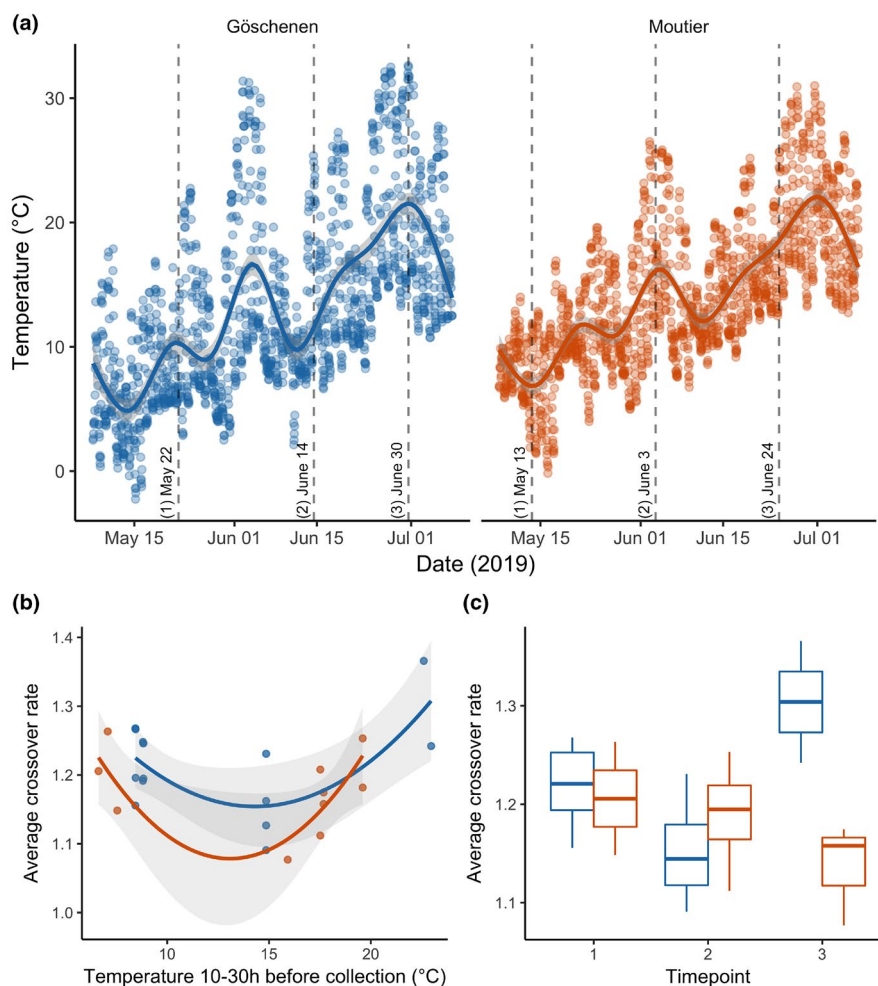


FIGURE 2 Hourly sampling of temperatures in summer 2019. (a) Hourly measurements of temperature (single points) at each plant from Göschenen are shown in blue and Moutier in orange. Smoothed lines show average trends for each site, with grey shading indicating the 95% confidence interval. Sampling timepoints at each site are shown with vertical dotted lines with dates and “campaign” numbers given in parentheses. (b) Average crossover rate per plant plotted against the temperature that that plant experienced averaged over the time window of 10–30 h before sampling. For correlations with temperature at individual timepoints, see Figure S1. Best fit polynomial curves are shown, with grey shading indicating the 95% confidence interval. Points and lines for GOS in blue and MOU in orange. (c) Box plots of average CO rates per plant for each population across the three sampling campaigns at each site with GOS in blue, and MOU in orange. Note that timepoint 3 in GOS has a small sample size

TABLE 1 Sample collection attributes within each study site, including the dates and durations of collection periods for each collection campaign, the range of temperatures during sample collection for each campaign, and resulting sample sizes after cytology for analysis

Site	Campaign	Date/time	°C	n Cells	n Ind.	% Cells w/ UV	% Cells w/MV
Göschenen	1	22.05.2019 11:10–12:55	7.8–10.8	110	8	17.27	24.55
Göschenen	2	14.06.2019 11:29–12:20	21.3	110	4	16.36	17.27
Göschenen	3	30.06.2019 12:36–13:40	31.5–32.3	8	2	12.5	12.5
Moutier	1	13.05.2019 11:43–15:05	10.5–13	24	3	0	45.83
Moutier	2	03.06.2019 11:55–13:41	23.5–28	33	4	18.18	27.27
Moutier	3	24.06.2019 10:35–12:00	18.5–24	39	3	25.64	35.9

Notes: Campaign corresponds to sample collection date for each site, and date/time gives the collection date and time. °C is the temperature range during collection. “n Cells” gives the number of meiocytes scored, and “n Ind.” the number of individual plants sampled. “% cells w/UV” is the percentage of cells that contain univalents and “% cells w/MV” is the percentage of cells that contain multivalents.

(see Methods). All scoring of metaphase I spreads was done initially blind to genotype or environmental factors to prevent biasing the results. We tested for correlations with the temperature in each site in the 30 h window prior to the collection time (Figure 2b; Figure S1), which was based on prior work showing the duration of meiosis in *A. thaliana* (Armstrong et al., 2003) and would fully encompass pre-meiotic S-phase and Prophase I in these plants. We found significant polynomial (“U-shaped”) relationships of per chromosome crossover rate with temperature in both MOU and GOS at multiple timepoints before sampling (Table 2; Figure 2b, Figure S1). The U-shaped response parallels results from controlled-condition laboratory studies of the closely related *A. thaliana* (Lloyd et al., 2018; Modliszewski et al., 2018). Since temperatures across the different timepoints are, to some extent, correlated (Figure S2), we cannot draw strong conclusions about the timing of temperature effects as one could from laboratory studies. We also tested for correlations with rainfall and developmental age (Figure S3); only temperature showed significant correlations.

GOS plants had significantly higher average per-chromosome recombination rates at all temperature timepoints except for 30 h prior to bud collection, with best model fits at the time of bud collection ($R^2 = .54$, $F = 5.58(4,19)$, $p = .038$) and at the 10–30 h average prior to bud collection ($R^2 = .53$, $F = 5.33(4,19)$, $p = .0047$) (Table 2, Table S2). Nevertheless, the response of recombination to temperature was very similar in GOS and MOU (Figure 2b, Figure S1). Presumably as a result of the link to temperature (although we cannot rule out all other factors, except developmental age and rainfall, which show no correlation with recombination rate), average recombination rates differed significantly across a season between both populations ($p = .032$, $F = 3.36 [4,18]$) (Figure 2c, Table S3), with GOS plants having higher CO rates than MOU plants at the beginning (comparatively cooler period) and at the end (comparatively warmer period) of the study. That the trends appear to differ between the populations may be due to small sample size of the last GOS timepoint (most plants had ceased flowering) and we thus cannot draw strong conclusions about the shape of the seasonal trend.

3.3 | Crossover rates and univalent versus multivalent formation

Low crossover rates could at least in theory cause some chromosomes not to receive any crossovers, which could cause univalent formation and thus chromosome mis-segregation in meiosis I. In autotetraploids such as *A. arenosa*, recombination rate increases could cause additional problems that diploids would not experience. This is because recombination rate correlates to some extent with the formation of multivalent associations among the multiple available pairing partners, which is in turn associated with meiotic segregation problems and can cause aneuploidy and reduced fertility (Bomblies et al., 2015, 2016; Grandont et al., 2013). We thus used the metaphase I images to estimate univalent and multivalent frequency in our samples, and tested for correlations with recombination rate and temperature.

We found that per cell univalent and multivalent frequency were indeed significantly correlated with crossover rate (Table 3, Figure 3a, Table S4). Univalents were rare overall, and did not show a significant general correlation with temperature (Figure 3b, Figure S4a). Lower crossover rates were nevertheless correlated with an increased number of univalents in both sites ($R^2 = .15$, $F = 29.01(2,321)$, $p < .001$), and univalents were almost exclusively detected in cells with low recombination rates. Multivalents showed a positive correlation with recombination rate in both sites ($R^2 = .09$, $F = 15.78(2,321)$, $p < .001$), but an increase in multivalents is much stronger at lower temperatures in both populations (Figure 3b, Figure S4b, Table 4). These responses differed significantly between GOS and MOS across all timepoints, with stronger multivalent responses in MOU than in GOS (Table S5, Figure S4b) and the best fitting model being at timepoint 20 h prior to bud collection ($R^2 = 0.59$, $F = 6.8(4,19)$, $p = .0014$; Table 4). As temperature warmed in each site through the study, the rate of multivalent formation differed significantly between MOU and GOS ($p = .012$, $F = 4.34(4,18)$), with MOU plants maintaining higher overall multivalent rates over time than GOS plants (Figure 3c, Table S3). Univalent rates, however, did not differ significantly across a season between both populations (Figure 3c, Table S3).

TABLE 2 Polynomial regression model outputs of the relationship between average crossover rate and each temperature period

Crossover rate model	Model equation	R^2	$F(df)$	p -Value
Temperature during collection	lm(Average Crossover Rate ~ poly(Collection Temperature, degree = 2, raw = T)):Site	.54	5.58 (4,19)	.0038
Temperature 30 h before collection	lm(Average Crossover Rate ~ poly(Temperature 30 h before collection, degree = 2, raw = T)):Site	.28	1.83 (4,19)	.16
Temperature 20 h before collection	lm(Average Crossover Rate ~ poly(Temperature 20 h before collection, degree = 2, raw = T)):Site	.47	4.25 (4,19)	.013
Temperature 10 h before collection	lm(Average Crossover Rate ~ poly(Temperature 10 h before collection, degree = 2, raw = T)):Site	.42	3.37 (4,19)	.03
Temperature 10–30 h before collection	lm(Average Crossover Rate ~ poly(Temperature 10–30 h before collection, degree = 2, raw = T)):Site	.53	5.33 (4,19)	.0047

Notes: Crossover Rate model gives the model used. Model Equation gives the equation. R^2 gives the R^2 value from the polynomial regression fit. $F(df)$ gives the F-value and in parentheses the degrees of freedom. The bold values indicate statistical significance ($p < 0.05$).

TABLE 3 Linear regression model outputs of univalent rate and multivalent rate as a function of crossover rate

Model	Model equation	R^2	$F(df)$	p -Value
Univalent rate	lm(Crossover Rate ~ Univalent Rate):Site	.1531	29.01 (2,321)	<.001
Multivalent rate	lm(Crossover Rate ~ Multivalent Rate):Site	.08953	15.78 (2,321)	<.001

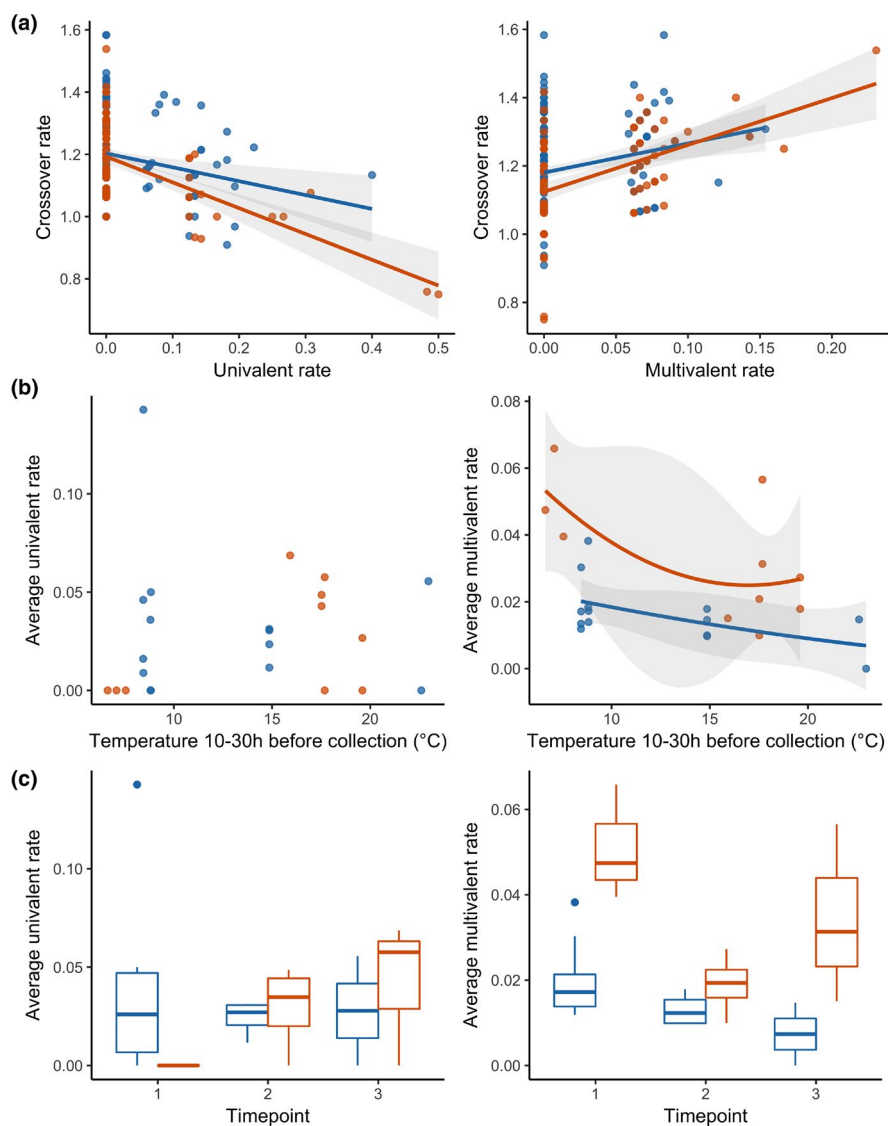


FIGURE 3 Univalent and multivalent formation. (a) Univalent (left) and multivalent (right) incidence per cell plotted against crossover rate measured for the same cell. Best fit linear regression fits are shown with grey shading indicating the 95% confidence interval. (b) Average univalent (left) and multivalent (right) rate per individual plotted against the temperature experienced by that individual averaged across the 10–30 h before sampling. There was no significant linear or polynomial trend for univalent rate, while multivalents showed a polynomial trend for MOU and a linear fit for GOS (grey shading indicating the 95% confidence interval). (c) Average univalent (left) and multivalent (right) rate per individual across the three campaigns. In all panels, Blue = GOS, orange = MOU

4 | DISCUSSION

We used two natural field populations of autotetraploid *Arabidopsis arenosa* to study whether genome-wide recombination rate, univalent rate, and multivalent formation rate vary across a growing season in nature. This was motivated by several prior pieces of information: (1) recombination rate, while well known to respond to temperature and to a lesser extent other environmental factors, has been studied almost exclusively in controlled laboratory conditions; (2) laboratory studies show that results vary by experimental design, suggesting effects may be complex or even canceled out in more variable field conditions; and (3) autopolyploids are predicted to suffer meiotic aberrations as recombination rate increases (see Introduction for more information). Our predictions were three-fold:

(1) We expected that, based on results from laboratory studies of *Arabidopsis thaliana* (Lloyd et al., 2018; Modliszewski et al., 2018), there would be a U-shaped relationship between growth temperature and recombination rate. (2) We expected that as recombination rates decline, univalent rates might increase, as this could increase that rate at which some chromosomes do not receive any CO events. (3) Because these plants are autotetraploids, and multivalent formation rates in polyploids are affected by recombination rate, we expected that as CO rates increase, multivalent rates should also increase. We found evidence to support all three predictions, although there were also some surprises.

First, we found that indeed recombination rates in the two genetically distinct populations showed a U-shaped correlation with temperature, with both lower and higher temperatures from a

TABLE 4 Polynomial regression model outputs of the relationship between average multivalent rates and each temperature period

Multivalent rate model	Model equation	R ²	F(df)	p-Value
Temperature during collection	lm(Average Multivalent Rate ~ poly(Collection Temperature, degree = 2, raw = T):Site	.45	3.89 (4,19)	.018
Temperature 30 h before collection	lm(Average Multivalent Rate ~ poly(Temperature 30 h before collection, degree = 2, raw = T):Site	.49	4.59 (4, 19)	.0092
Temperature 20 h before collection	lm(Average Multivalent Rate ~ poly(Temperature 20 h before collection, degree = 2, raw = T):Site	.59	6.80 (4,19)	.0014
Temperature 10 h before collection	lm(Average Multivalent Rate ~ poly(Temperature 10 h before collection, degree = 2, raw = T):Site	0.55	5.78 (4,19)	.0032
Temperature 10–30 h before collection	lm(Average Multivalent Rate ~ poly(Temperature 10–30 h before collection, degree = 2, raw = T):Site	0.53	5.33 (4,19)	.0048

mid-point correlating with higher recombination rates. This is in line with previous work in controlled laboratory conditions for *A. thaliana* (Lloyd et al., 2018; Modliszewski et al., 2018) and a wide range of other eukaryotes (see for review: Bomblies et al., 2015). Importantly, this result shows that the U-shaped curves that have been observed in laboratory studies are also observed in the field, despite the much more strongly and unpredictably fluctuating temperatures. This is reassuring as it suggests laboratory studies can informatively complement field studies of recombination rate plasticity. Since temperatures across the time series correlate to some extent, we cannot use this to precisely identify the most relevant timepoints or define the exact “low point” temperature, but it is interesting to note that in the 10–20 h before collection the plants should be in approximately early prophase I (Armstrong et al., 2003), which is when crossovers are initiated and maturing, and is known to be a particularly sensitive stage for temperature effects on meiosis (De Storme & Geelen, 2020; Draeger & Moore, 2017). We also tested for a correlation with developmental age, which was previously shown in *A. thaliana* to correlate with changes in recombination rate (Li et al., 2017; Toyota et al., 2011), but there was no correlation in our data set. Finally, since rainfall is another environmental factor that affects plant growth and stress, we also tested whether it is correlated with recombination rate, but found no correlation. We also note that we cannot rule out day length being an additional factor, as like temperature, it increases throughout the sampling period, and day length has also been implicated in causing recombination rate variation in *A. thaliana* (Boyko et al., 2005).

Although they are genetically distinct, and GOS plants had on average higher recombination rates throughout the study, GOS and MOU show similar plastic correlations of recombination rate with temperature. This suggests that despite their divergence in average recombination rate, the plasticity of recombination rate to temperature is largely unchanged. This observation parallels results from laboratory evolution studies in *Drosophila* where lines experimentally evolved in different temperature regimes show differences in recombination rate, but not in plasticity (Kohl & Singh, 2018), and laboratory-selected populations for adaptation to desiccation stress also showed divergence in overall genome-wide recombination rate upon exposure to stress, but similar plasticity (Aggarwal et al., 2019).

We found that in these autopolyploid populations, plasticity has at least some cost in that both high and low recombination rate gametes show higher rates of meiotic aberrations that can cause chromosome segregation problems. First, at temperatures where recombination rates are their lowest, cells are more likely to have unpaired univalents, though we did not observe high univalent rates in any samples. This could occur if meiosis is not able to reliably maintain the minimum “obligate” crossover required for proper chromosome segregation in anaphase I (Hunter, 2015; Jones & Franklin, 2006), or, in the polyploid context, if some chromosome sets resolve into trivalent/univalent combinations (Bomblies et al., 2016). This suggests that when CO rates drop in response to environment, the risk of univalent formation, which can lead to aneuploidy, increases. Second, and perhaps more importantly, autopolyploids, like the sampled *A. arenosa* populations, also run a risk as recombination increase that diploids do not experience. This is because increased recombination is predicted to lead to increased formation of multivalents (Bomblies et al., 2015, 2016; Grandont et al., 2013). Multivalents occur when the multiple copies of each homologous chromosome recombine with more than one partner, and have been associated with increased rates of chromosome mis-segregation in polyploids (Bomblies et al., 2016). Reducing crossovers to one per chromosome is a potentially effective way of preventing multivalents, and an increase in temperature could thus theoretically increase multivalent rates. We tested for this here, and indeed, we found that in these autotetraploid *A. arenosa* populations, multivalent frequency per cell correlated positively with recombination rate. This suggests that, as predicted (Bomblies et al., 2015), environmentally-driven recombination rate deviations from some optimum could lead to increased meiotic instability in autopolyploids due to the formation of multivalents, which could mean that autopolyploids may disproportionately face fertility or genome stability challenges as temperatures fluctuate in natural populations. Considering that autopolyploids are quite common in nature, especially among plants (Barker et al., 2016; Soltis et al., 2007), this may become a significant issue for these species as climates change.

The correlation of multivalents with temperature showed an interesting feature that is not quite what the overall significant positive correlation between recombination rate and multivalent rate would predict. In both MOU and GOS, it seems to matter whether

recombination rate increases are associated with high temperature or low temperature. Though both populations have a U-shaped curve with recombination rate apparently increasing about equally in low and high temperatures, both populations show an increase in multivalent formation only (or mostly) at lower temperatures. This suggests it is not recombination rate, or at least not only recombination rate, that determines multivalent rate. The observation that multivalents increase particularly in the cold is interesting in light of previous work on recombination rate plasticity in *A. thaliana*. It was shown previously that both low and high temperatures cause recombination rate increases via an effect on Class I (interference-sensitive) crossovers (Lloyd et al., 2018; Modliszewski et al., 2018). However, in low but not high temperatures there is also an associated increase in length of the chromosomal axes (Lloyd et al., 2018). The chromosome axes and synaptonemal complex are long linear structures that form along the chromosomes during meiosis, whose length correlates well with recombination rate in many species (Lynn et al., 2002; Ruiz-Herrera et al., 2017; Wang et al., 2019; Wang, Veller, et al., 2019). Chromosome axis length has also been previously positively correlated with multivalent formation in *A. arenosa* (Morgan et al., 2020). Thus, it may be that an increase in chromosome axis length at low temperature is responsible both for the increase in recombination rate and higher multivalent formation seen here, while the increase in recombination at high temperature, where the axis shortens further, at least in *A. thaliana* (Lloyd et al., 2018), is not similarly conducive to multivalent formation. How this might work mechanistically is not yet obvious. This result does, however, imply that the recombination rate increases at low and high temperatures are at least somewhat mechanistically distinct, and that multiple factors may be at work, at least with regard to multivalent formation. This hints at a not yet understood link between axis structure and multivalent formation rates in autopolyploids.

The observation that recombination rates vary across a season in natural populations has interesting implications. For plants like *A. arenosa*, early and late in a season, temperatures are likely to deviate above and below a particular optimum (which will vary among species). This means that early and late season gametes are more recombined on average than those produced midseason due to what are probably unavoidable biophysical effects of temperature on meiosis, that is, that meiosis becomes progressively compromised as temperatures deviate from a mid-range (Morgan et al., 2017). This plasticity also has some costs – the least recombined gametes produced mid-season have a higher (although never high) rate of univalent formation, which would apply also in diploids, while the most recombined gametes (produced at lower temperatures) have a higher rate of multivalent formation, a challenge unique to polyploids. Whether or how these effects influence the genetics of these populations, or their adaptability, remains to be tested.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Kirsten Bomblies and Andrew P. Weitz designed the study, analysed metaphase spread images, and wrote the manuscript. Andrew P. Weitz performed field and laboratory research, and analysed the data. Marinela Dukic contributed essential methodological training and helped with cytology, and helped write the manuscript. Leo Zeitler generated and analysed the population genetic data and helped write the manuscript. All authors helped edit the manuscript.

DATA AVAILABILITY STATEMENT

Metaphase images and scoring are available through the ETH research collection with <https://doi.org/10.3929/ethz-b-000471742>. Sequencing data are available in the NCBI SRA under accession SRP268902.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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